

The intake of lead and associated metals by sheep grazing mining-contaminated floodplain pastures in mid-Wales, UK: I. Soil ingestion, soil–metal partitioning and potential availability to pasture herbage and livestock

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abstract

This paper first evaluates the relative importance of the soil–plant–animal and soil–animal pathways of Zn, Cu and (especially) Pb investigated over a 15-month study period at 12 floodplain sites located within and downstream of the mineralised and historic mining area of mid-Wales, and secondly considers the implications of a sequential extraction procedure (SEP) undertaken on soils of varying particle size sampled from the study locations. Generally, very good agreement was found between the chemical partitioning of the three metals for each of the physical soil fractions subjected to the SEP. The availability of Pb to pasture vegetation, especially at the contaminated sites, is indicated with its associations with the more soluble (i.e. exchangeable and Fe/Mn oxide) soil phases, yet soil and/or plant barriers effectively restrict above-ground herbage concentrations of this metal. Consequently, with sheep ingesting soil at rates varying according to season from 0.1% to 44% or more of dry matter intake, the soil–animal pathway accounts for the majority of Pb consumption through most of the year, and at moderately and highly contaminated sites significant quantities of relatively soluble soil–Pb can be ingested at rates exceeding safety threshold limits.

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1. Introduction

Historical mining, the weathering of mineral ores and subsequent fluvial redistribution across mid-Wales, has led to the deposition of contaminated sediments and enrichment of downstream floodplain soils with metals including zinc (Zn), copper (Cu) and especially lead (Pb; Alloway and Davies, 1971; Davies and Lewin, 1974; Lewin et al., 1977). Although all mining activity in the area had ceased by 1942, fluvial re-working and the erosion, transport and deposition of dissolved and suspended constituents from mine-workings, spoil heaps and river banks remain a contemporary source of floodplain soil–metal contamination (Davies, 1987; Lewin and Macklin, 1987). Deposited alluvium typically creates a fertile soil heavily used for agricultural purposes, although in mid-Wales problems of metal contamination can occur for a great distance downstream of the original mining/mineralisation source. Towards the end of the N2000 year mining history Griffith (1919) reported toxic effects to fish in the rivers draining these mineralised catchments, and to crops and livestock on associated floodplains. Incidents of Pb intoxication in cattle as a result of exposure to high concentrations in soils and mining spoil have been reported more recently in the same area (e.g. VLA, 2008), and an initial reconnaissance survey and metal analysis of

soils, pasture herbage and sheep faeces was summarised by two of us (Abrahams and Steigmajer, 2003), who identified the importance of ingested soils in supplying metals, especially Pb, to grazing livestock. Our initial survey period of investigation has subsequently been extended over 15 months and soil ingestion rates have been recalculated using improved analytical techniques. This paper firstly reports on the relative importance of the soil–plant–animal and the soil–animal pathway of Zn, Cu and Pb to sheep grazing the floodplain pastures of mid-Wales over the 15 month study period. Secondly, we consider the results of a widely known sequential extraction procedure (SEP) employed on soil samples collected from the study fields. The SEP estimates soil–metal partitioning, and we: (a) compare the results from the procedure undertaken on varying soil particle size fractions, and (b) consider the implications for potential soil–metal availability to pasture plants and sheep grazing the floodplain herbage.

2. Materials and methods

2.1. The study area

The Central Wales mining district is recognised as a complex orefield in which multiple phases of mineralisation involving Pb, Zn, Cu and other elements such as cobalt and nickel have occurred (Mason, no date). Mining and the processing and smelting of ores

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have taken place in the region since Bronze Age times when Cu production was undertaken (Mighall et al., 2002). During the period of the Roman Empire there was a focus on Pb mining that is also evident later in Medieval times and from the 16th century until the early 20th century. Zinc output became important from the mid-19th century onwards. Whilst minerals such as galena (PbS, the most important primary metalliferous mineral found within the study area) can be recognised as being associated with supplying low concentrations of available Pb to plants/animals, the chemical weathering of such constituents in soils has a significant influence on metal availability to organisms. Thus, Mason (2004) notes the widespread occurrence of secondary cerussite (PbCO_3) and pyromorphite [$\text{Pb}_5(\text{PO}_4)_3\text{Cl}$] formed as a result of the chemical weathering of PbS: the latter is a stable soil–Pb mineral of extremely low solubility (and hence availability), whilst in comparison the former is associated with more readily available Pb to organisms (Plumlee, personal communication).

Fieldwork was undertaken in mid-Wales on the floodplains of four rivers (Afon Clarach, Rheidol, Teifi and Ystwyth) historically affected by metalliferous mining activity. The Afon Aeron was additionally identified as a control catchment. In all, twelve sample sites were selected from within the five catchments, representing the total soil Pb concentrations found across the area (see Abrahams and Steigmajer (2003) for details), and grouped into three categories: 'uncontaminated or control soils with $\leq 150 \mu\text{g Pb g}^{-1}$ (sites U1–4)', 'moderately contaminated soils containing $150\text{--}1000 \mu\text{g Pb g}^{-1}$ (sites M1–5) and 'highly contaminated' soils with $\geq 1000 \mu\text{g Pb g}^{-1}$ (sites H1–3). All soils are associated with alluvium or gravel terraces and belong to the Conway (poorly drained gley soils on silty alluvium), Rheidol (moderately well drained brown earths on terrace gravels) or Clwyd (an imperfectly drained intergrade lying between the former soils) series (Rudeforth, 1970) that typically have topsoils which are slightly to moderately acidic in reaction (topsoil pH at the 12 study sites measured in a 1:2.5 w/v CaCl_2 suspension varied from 4.5–6.3, median = 5.2) and contain low/medium amounts of soil organic matter (4.5–8.9%, median = 6.0%; Steigmajer (1999)). Each site was under pasture and known to be regularly grazed by sheep. The sampling areas were restricted to that available for grazing and were thus determined by field and farm boundaries. Sites were selected specifically where the majority of that boundary lay on the floodplain, directly adjacent to the river and so affected by recurrent flood events and depositions of river material including historical mining-related contamination.

2.2. Method for evaluating the soil–plant–animal and soil–animal pathways of Pb, Zn and Cu

With fields ≥ 4 ha in area, a 3.5 cm diameter screw auger was used to collect (from a 'W' shaped traverse walked at each site) a representative topsoil sample by bulking at least 25 0–15 cm depth cores. Following preparation, samples were analysed for their pseudo total metal content utilising a $\text{HNO}_3\text{--HClO}_4$ digestion. Above-ground pasture vegetation and the faeces of sheep were routinely collected from the fields every other month over a period of 15 months from January 1999 to March 2000. Vegetation was cut using Teflon-coated stainless steel scissors, taking care to avoid pulling up the root mass, and where possible, removing only that above 1 cm from the soil surface. A 'W' traverse was utilised to obtain a bulked representative sample collected from at least 25 points. Mixed pasture herbage samples were collected, but no attempt was made to evaluate species composition. Faecal matter was collected with a stainless steel trowel, avoiding any that had been in obvious contact with the soil surface. Faeces were taken from at least 20 different specimens in each field, and bulked to form a representative sample. Many details regarding preparation and analysis of these samples have been previously reported by Abrahams and Steigmajer (2003) and for the sake of brevity are not repeated here. In the laboratory vegetation samples

were divided into two: the first left unwashed to reflect any contamination with soil particles in the field environment and the second, representing metal uptake from soils and translocation to the above-ground portion of the plant, thoroughly washed three times in deionised water, or until the washings were free from visible soil particles. The inefficiency of washing has been previously cited by Fortmann and Johnson (1984) as a problem in interpreting the results from such a preparation procedure. Subsequent comparison of both washed and unwashed vegetation samples in our work, using titanium (Ti) as a stable marker of the constituent proportion of soil particles, indicated an efficiency of the washing procedure between 60 and 93%. On this basis the recorded concentrations of metals in washed herbage may be an over-estimation of that actually present in the plant tissues, attributable to contamination with soil particles enriched in these elements.

Soil ingestion by grazing sheep was estimated from the Ti concentrations of soils and faecal material. The samples were digested using a $\text{HCl--HClO}_4\text{--HF}$ procedure and Ti^{47} concentrations (this isotope was preferred as it displayed a greater precision and accuracy under analysis and has no known spectroscopic interferences) were determined by inductively coupled plasma-mass spectrometry (ICP-MS) using a VG Elemental PlasmaQuad II instrument with Ru^{102} as an internal calibration standard (Smith, 2004). Soil ingestion rates were estimated as a percentage of dry matter (DM) intake according to the formula of Thornton and Abrahams (1983) and assuming pasture digestibility of 72% (Green et al., 1996):

$$k \text{ soil ingestion} = \frac{\delta I - D_h \text{Ti}_f \times 100}{\text{Ti}_s - D_h \text{Ti}_f} \quad \delta I \text{P}$$

Where D_h is the digestibility of the vegetation, Ti_s is the Ti concentration of the soil (minimum–maximum = $1544\text{--}3827 \mu\text{g g}^{-1}$ in our study, median = $3316 \mu\text{g g}^{-1}$) and Ti_f is the Ti concentration of the faeces ($19\text{--}2365 \mu\text{g g}^{-1}$, median = $740 \mu\text{g g}^{-1}$).

2.3. Method for the sequential extraction procedure

Following collection of the representative topsoils, the samples were air-dried, (as recommended in the Community Bureau of Reference (BCR) standardised sequential extraction procedure (Quevauviller et al., 1993a,b; Ure et al., 1993)) in the laboratory and stored at 4°C , to reduce further microbial action on the form of soil–metals, until required. Particle size is important in laboratory analyses (Salomons and Förstner, 1984) as it is considered to be a factor governing both the total concentration of soil–metals and also their potential bioavailability (e.g., Macklin and Dowsett, 1989). Whilst the $\geq 2000 \mu\text{m}$ 'fine earth' fraction is often cited to be geochemically reactive and used in bioavailability research (Quevauviller et al., 1993a; Hudson-Edwards et al., 1996; Dawson and Macklin, 1998), others have advocated the use of finer fractions: $\geq 1000 \mu\text{m}$ (Davidson et al., 1998; Zhang et al., 1998), $\geq 170 \mu\text{m}$ (Li and Thornton, 2001; Maskall and Thornton, 1998) and $\geq 63 \mu\text{m}$ (Bird et al., 2003; Bradley and Cox, 1987; Ho and Evans, 2000; Ure et al., 1993). In our research, following an initial analysis undertaken on the $\geq 2000 \mu\text{m}$ fine earth, the sequential extraction procedure was additionally performed on the $\geq 63 \mu\text{m}$ (silt and clay) and $\geq 63\text{--}2000 \mu\text{m}$ (sand) constituent particle sizes to assess the variability in metal partitioning and the reliability of data from analysis solely of the $\geq 2000 \mu\text{m}$ 'reactive' fraction reported in previous studies. Air-dried soil samples were disaggregated using an acid-clean agate pestle and mortar prior to being passed through a stainless steel sieve stack to collect the $\geq 63 \mu\text{m}$, $63\text{--}2000 \mu\text{m}$ and $\geq 2000 \mu\text{m}$ fractions. Samples for each particle size were thoroughly homogenised before sequential extraction.

The SEP used was adapted from that designed and developed by the BCR (Community Bureau of Reference; now the Standards,

Measurements and Testing Program of the European Commission; Ure et al., 1993) due to its wide application in contaminated soil and sediment research (e.g. Alvarenga et al., 2008; Bird et al., 2003; Ho and Evans, 2000; Larner et al., 2008), and its progress through several years of directed development, harmonisation and improvements to address concerns of quality control (López-Sánchez et al., 1993; Quevauviller et al., 1993a,b; Salomons, 1993; Ure et al., 1993). Briefly, the sequential chemical phases of extraction are: (1) 0.11 M acetic acid solution associated with exchangeable and carbonate-bound metals, (2) 0.5 M $\text{NH}_2\text{OH} \cdot \text{HCl}$ associated with reducible (oxy)hydroxides of Fe and Mn, (3) 8.8 M H_2O_2 and 1.0 M ammonium acetate associated with oxidisable organic matter and sulphides and, (4) aqua regia to determine the metal content of residual minerals.

Lead, Zn and Cu concentrations were determined by flame atomic absorption spectrophotometry (AAS) using a Perkin-Elmer 2380 instrument with an air/acetylene flame/burner. The results presented in our manuscript are given in, or have been corrected for, the dry weight of the material after oven-drying at 105 °C until constant weight.

2.4. Analytical quality control (AQC) procedures

To evaluate the robustness of our data we used seven certified reference materials (of vegetation, soil or sediment) to measure the accuracy of our laboratory analysis and, as an internal check on the accuracy of the SEP, the total amount of each metal extracted (i.e., sum of phases 1 + 2 + 3 + 4) was compared with that obtained by aqua regia digestion of a separate subsample of the soil, as recommended in the BCR procedure (Ure et al., 1993). In addition, procedural blank samples were used to confirm that metal contamination was not a significant source of error, and sample replicates to assess the precision of analysis. Samples, reference materials, blanks and replicates were analysed blind and in a random order to minimise any bias associated with instrumental drift and human subjectivity. Instrumental calibration standards were made up in each of the matrices from the SEP and the other digestion procedures in order to minimise matrix effects. Our findings using these various matrices, and the results of our AQC procedures, have been fully outlined by Smith (2004).

3. Results

3.1. The soil–plant–animal and soil–animal pathways of metal contaminants

Our initial reconnaissance survey indicated a seasonal variation in the Pb concentrations of washed pasture herbage, and high rates of soil ingestion by sheep during the winter months (Abrahams and Steigmajer, 2003). These early observations are confirmed over the longer timescale of the 15 month study period. For example, Fig. 1 illustrates the seasonal variation in pasture plant–metal concentrations at one of the study sites. The washed vegetation Pb concentrations at this location ranged from $33 \mu\text{g g}^{-1}$ in July to $700 \mu\text{g g}^{-1}$ in March (though note, considering the inefficiency of the washing procedure highlighted in the methodology, the actual above-ground plant tissues of washed herbage samples are likely to contain lower concentrations than those illustrated in Fig. 1). The seasonal pattern of accumulation in the washed above-ground pasture species is also evident for Zn and Cu, and calculation of relative accumulation ratios (estimated from the ratio of concentrations in washed vegetation and soil; Timperley et al., 1970) indicates that the uptake and translocation of these two metals is greater compared to Pb. Thus, the relative accumulation ratios for Pb were in most cases ≤ 0.08 , whereas the median accumulation ratios for Cu and Zn throughout the 15 month study were 0.23. While significant ($P < 0.05$) Spearman rank correlation coefficients show that washed pasture herbage Pb concentrations

reflect the total soil content throughout the study period (similar significant associations are also evident for Zn in July and September and for Cu for most of the year), these results show that barriers are operating to limit uptake into the above-ground pasture tissues, especially for Pb. The relative accumulation ratios were markedly lower in the summer months. For example ratios for Pb ranged from 0–0.04 in July and 0.01–0.25 in January. This trend reflects the general decline in washed plant–metal concentrations assumed to be associated with the increase in pasture biomass in the summer months and the relative dilution of tissue metal concentrations.

Throughout the year the concentration of metals in unwashed pasture samples were generally elevated over their washed counterparts, with a seasonal trend of elevation over the winter and reduction during the summer growing season. The highest unwashed herbage concentrations recorded were $1350 \mu\text{g Pb g}^{-1}$, $350 \mu\text{g Zn g}^{-1}$ and $49 \mu\text{g Cu g}^{-1}$ in March (1999) at the site with the highest levels of soil–Pb contamination (Fig. 1). The seasonal pattern is attributed in part to an increased susceptibility of pasture herbage to soil contamination in the winter months. When soils are wetter (note: the ‘average’ annual precipitation varies according to site from ca. 100–180 cm, with October/November generally being the wettest months and April/May/June the driest (Rudeforth, 1970)), and pasture biomass and height are decreased, trampling (poaching) and rain splash are more likely to transfer soil

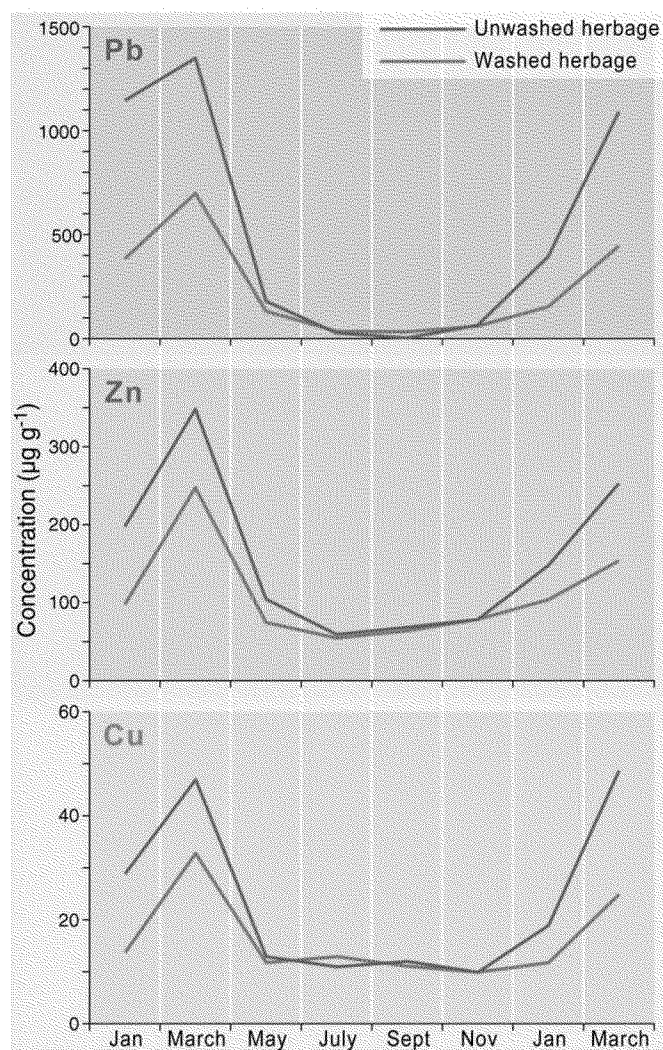


Fig. 1. Seasonal variation in washed and unwashed pasture plant–metal concentrations at the site with highest Pb contamination (H3): soil contained pseudo total concentrations of $2940 \mu\text{g Pb g}^{-1}$, $252 \mu\text{g Zn g}^{-1}$, and $76 \mu\text{g Cu g}^{-1}$.

Table 1

Seasonal variation of soil ingested by sheep grazing pastures in mid-Wales (the rates of soil ingestion are determined using Eq. (1) presented in the text).

Sample period		n	Percentage of soil as DM intake	
Month	Year		Median	Minimum–maximum
January	1999	11	15.1	4.9–67.9
March		11	12.8	4.3–44.0
May		10	4.1	1.5–9.3
July		8	1.5	0.7–2.7
September		9	0.4	0.1–1.7
November	2000	11	3.8	1.0–22.8
January		10	11.6	1.5–24.1
March		12	11.8	2.3–81.8
Overall		82	7.6	0.1–81.8

Several variables influence how much soil is consumed – e.g. soil characteristics such as drainage status; management practices that control stocking rate – with factors such as the vulnerability of pasture to soil contamination during the winter accounting for the observed maximum rates of ingestion during this period.

particles onto the surfaces of vegetation. In contrast, throughout the summer growing season the soils are drier and plant vulnerability to soil contamination decreases as the biomass increases.

The susceptibility of soil contaminated herbage during the winter months is coincident with elevated rates of soil ingestion by grazing sheep (Table 1). The maximum rate of 81.8% DM intake recorded at site H2 in March 2000 is echoed by the next highest estimation (67.9%) recorded at the same location the previous year. While such high rates of soil ingestion are surprising and appear anomalous, we have no reason to believe that they are not accurate and observation of the pasture sward at the time of sampling of this site indicated a heavy contamination of adhering soil particles. However, at all other times of sampling, and at other sites, soil was found to constitute less than half of the total DM intake. Thus, the two high values aside, soil ingestion rates by sheep varied between 0.1 and 44.0% DM intakes as typically

reported elsewhere (Beresford and Howard, 1991; Healy, 1967; Suttle et al., 1975).

The total daily metal intakes by sheep were calculated by assuming a dietary consumption of 1.0 kg DM d⁻¹ (as recommended by Adesogan (personal communication) following McDonald et al. (1995)), equivalent to 2.5% of a 40 kg animal, and estimates are given in Table 2. For the low soil–Pb sites total intake of this metal by sheep did not exceed 25 mg d⁻¹ in winter; well below the safety threshold limit recommended by the ARC (1980) of 2 µg g⁻¹ live weight d⁻¹ or an ingestion of 60–100 mg d⁻¹. However, this safe limit was exceeded in the winter months at 3 of the sites with moderate soil–Pb contamination and throughout the year at locations M5 and H1-3. The highest Pb intake of 1685 mg d⁻¹ – almost 17 times the recommended limit – was recorded at the most contaminated site. Following the seasonal pattern of soil contaminated vegetation and soil ingestion rates, the highest Pb intakes occurred in the winter months. For the most contaminated site the median winter Pb intake was 783 mg d⁻¹, while in summer at this location the median Pb intake was reduced to 55 mg d⁻¹. The principal source of Pb intake was found to be by the direct ingestion of soil which accounted for more than half of ingested Pb for most of the year. For six of the eight sampling periods studied over 90% of Pb intake at some sites was via soil ingestion, with an overall median across all sites and seasons of 66% Pb ingested as soil.

The ARC (1980) suggests that a diet comprising 150 mg Zn d⁻¹ is well tolerated and, in general, ruminant endurance to Zn intake is thought to be good. Adverse effects of Zn ingestion can become apparent in the range 150–420 mg d⁻¹ which was exceeded in two instances in this study, although at concentrations indicative of mild effects rather than chronic risk as the excessive intakes were limited to a short winter period. Limited daily intake rates for Cu (6–60 mg d⁻¹) indicate that toxicity associated with this metal is unlikely to be a problem for sheep grazing these floodplain sites. Although at times over 90% of Zn and Cu may be ingested in the form of soil (Table 2), direct soil ingestion is not the major source of intake for grazing sheep, with an overall median of 18% intake as soil for both metals. The

Table 2

The minimum–maximum (and median in brackets) soil and washed pasture herbage metal concentrations (µg g⁻¹), calculated daily soil, herbage and total metal intakes (mg d⁻¹) for sheep^a, and percentage of metal ingested as soil.

	Soil concentration			Washed pasture herbage concentration			Daily intake as soil		
	Pb	Zn	Cu	Pb	Zn	Cu	Pb	Zn	Cu
January 1999	46–2940 (588)	84–546 (210)	17–76 (25)	3–385 (60)	45–250 (100)	8–14 (9)	2–1312 (77)	6–371 (28)	1–48 (4)
March				2–700 (31)	45–250 (95)	9–33 (11)	3–1292 (68)	7–111 (27)	1–33 (4)
May				b1–130 (6)	40–80 (53)	7–12 (10)	1–117 (8)	2–33 (10)	b1–4 (1)
July				2–39 (6)	30–95 (50)	7–13 (10)	1–51 (2)	1–15 (3)	b1–2 (1)
September				1–48 (3)	30–220 (46)	6–12 (10)	b1–34 (1)	b1–9 (1)	b1–1 (b1)
November				2–60 (5)	30–200 (55)	7–10 (9)	b1–441 (17)	1–125 (9)	b1–16 (2)
January 2000				2–155 (17)	40–150 (63)	10–13 (11)	2–437 (40)	3–42 (21)	b1–14 (3)
March				2–450 (18)	40–215 (78)	9–25 (11)	5–1580 (73)	5–446 (25)	b1–58 (4)
	Daily intake as pasture			Total daily intake			% ingested as soil		
	Pb	Zn	Cu	Pb	Zn	Cu	Pb	Zn	Cu
January 1999	3–298 (34)	50–142 (82)	3–11 (9)	5–1349 (163)	63–451 (127)	8–51 (12)	31–97 (66)	6–82 (35)	10–94 (35)
March	2–392 (28)	39–144 (73)	7–18 (10)	6–1685 (99)	50–251 (130)	10–52 (14)	22–91 (77)	5–54 (26)	8–64 (31)
May	b1–17 (6)	37–73 (51)	8–12 (10)	3–133 (14)	43–104 (55)	8–13 (11)	32–100 (73)	3–39 (15)	4–36 (10)
July	2–33 (2)	30–74 (42)	8–13 (11)	3–60 (4)	31–88 (45)	8–14 (12)	22–87 (43)	3–17 (5)	2–16 (4)
September	2–34 (2)	30–216 (45)	6–12 (10)	1–81 (3)	30–226 (47)	6–13 (10)	9–42 (32)	1–5 (2)	b1–9 (1)
November	2–58 (3)	29–154 (48)	6–10 (8)	2–447 (22)	31–279 (65)	7–22 (10)	20–99 (79)	4–45 (23)	3–72 (18)
January 2000	2–132 (14)	38–131 (51)	8–11 (10)	4–569 (63)	43–152 (81)	10–23 (12)	35–93 (63)	7–48 (27)	3–63 (26)
March	2–390 (14)	36–134 (62)	2–22 (9)	6–1592 (84)	49–486 (100)	9–60 (14)	49–99 (76)	10–92 (28)	5–97 (28)

^a Assuming a diet of 1 kg DM d⁻¹ (Adesogan, personal communication) and applying rates of soil ingestion reported in this study (Table 1). A specific example (site H3, January 1999) is discussed here to show how these calculations are made: This site has a soil concentration of 2940 µg Pb g⁻¹ (= 2.94 mg g⁻¹), and during January sheep were ingesting soil at a rate of 22.6% (= 226 g of the 1 kg diet). Thus, the daily Pb intake as soil = 2.94 × 226 = 664.4 mg d⁻¹. The remaining 774 g of the diet is comprised of herbage containing 385 µg Pb g⁻¹ which equates to a daily intake as pasture = 298 mg d⁻¹.

higher relative accumulation of these metals by pasture plants indicates that in the majority of cases the indirect soil–plant–animal pathway is the principal, and limiting, route of exposure.

3.2. The SEP and total metal concentrations of the b63 μm (silt and clay), 63–2000 μm (sand) and b2000 μm soil particle size fractions

In general the b63 μm soil fraction was found to have higher total Zn and Cu concentrations than the 63–2000 μm (sand) fraction, although a less consistent pattern was evident for Pb (Fig. 2). Lower concentrations of Pb were generally found for the b2000 μm fine earth fraction, than either of its constituent fractions, and this was often the case for Zn and Cu also. These variations may be attributable to an extent to the poorer analytical precision associated with the b2000 μm fraction that we noted from our AQC procedures, perhaps

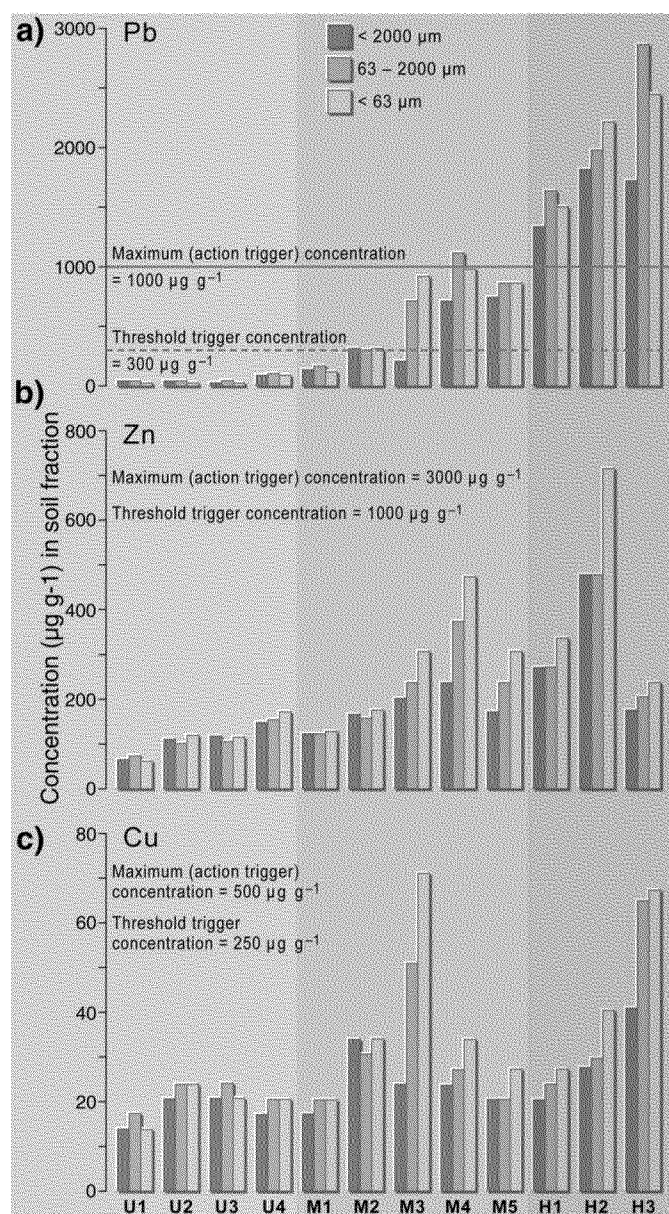


Fig. 2. Total soil–metal concentrations according to physical fraction. The maximum or action trigger soil concentration for grazing livestock is that above which there is a very high probability of zootoxic effects which may result in the death of farm animals. The threshold trigger concentration is defined as that below which the soil is considered to be safe (ICRCL, 1990). Between the 2 trigger concentrations sub-clinical toxicity may affect livestock. Using such guidelines, irrespective of particle size the soils studied can be considered safe in terms of their Cu and Zn content.

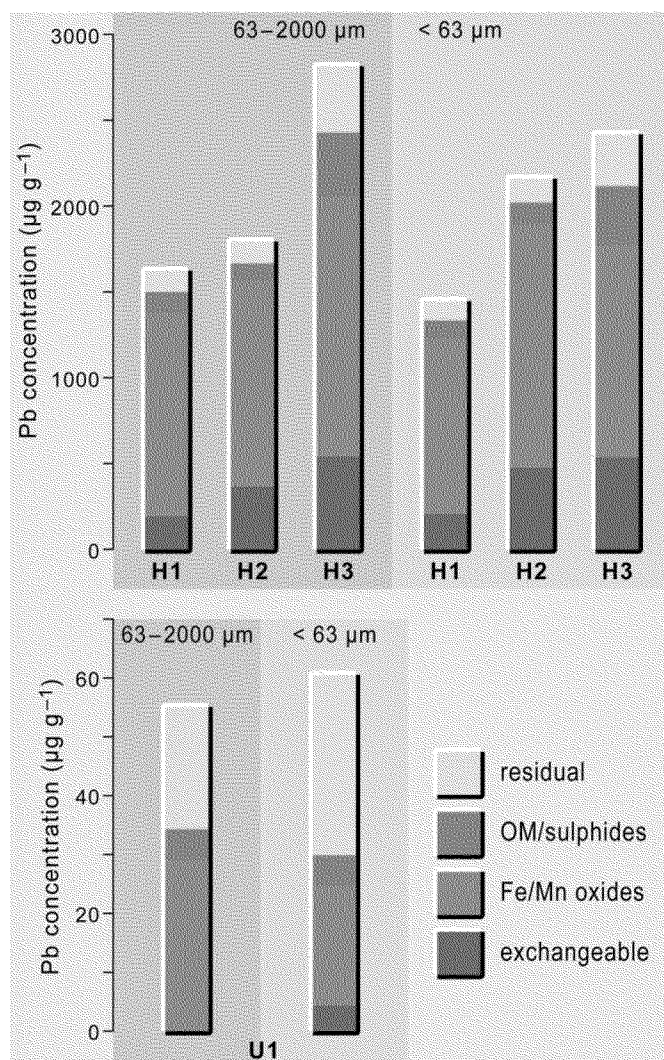


Fig. 3. Comparison of soil Pb partitioning between physical fractions for the highly contaminated sites. Control soil U1 is shown for comparison.

attributable to difficulties in ensuring a homogeneous sample. The results indicate that it may not be sufficient to analyse the b2000 μm fraction only, as it does not always provide a full insight on the total soil–metal concentrations which can vary according to its constituent particle sizes. Individual analysis of either the b63 μm or 63–2000 μm fractions can also be problematic, since variability of total metal concentrations according to the soil particle size analysed can have implications with the interpretation of the results when comparing to environmental guidelines and classification of contamination levels. For example, the ICRCL (1990) ‘maximum or action trigger’ and ‘threshold trigger’ concentrations, which have been specifically applied to mining-contaminated soils in the UK where a risk may be posed to grazing livestock, are shown in comparison with the total Pb concentrations across the study area in Fig. 2a. The three most contaminated sites (H1–3) clearly exceed the given action trigger threshold for soil Pb – and all concentrations associated with site M5 are greater than the threshold trigger concentration – irrespective of particle size, but at two sites (M3, M4) whether the ICRCL guidelines are exceeded depends on the particle size considered.

Generally very good agreement was found between the partitioning of Pb, Zn and Cu for each of the physical soil fractions subjected to the SEP procedure (e.g. Fig. 3). Evident discrepancies occurred only in the control soils with concentrations at the limits of detection. The partitioning of Pb, Zn and Cu from the SEP undertaken on soils

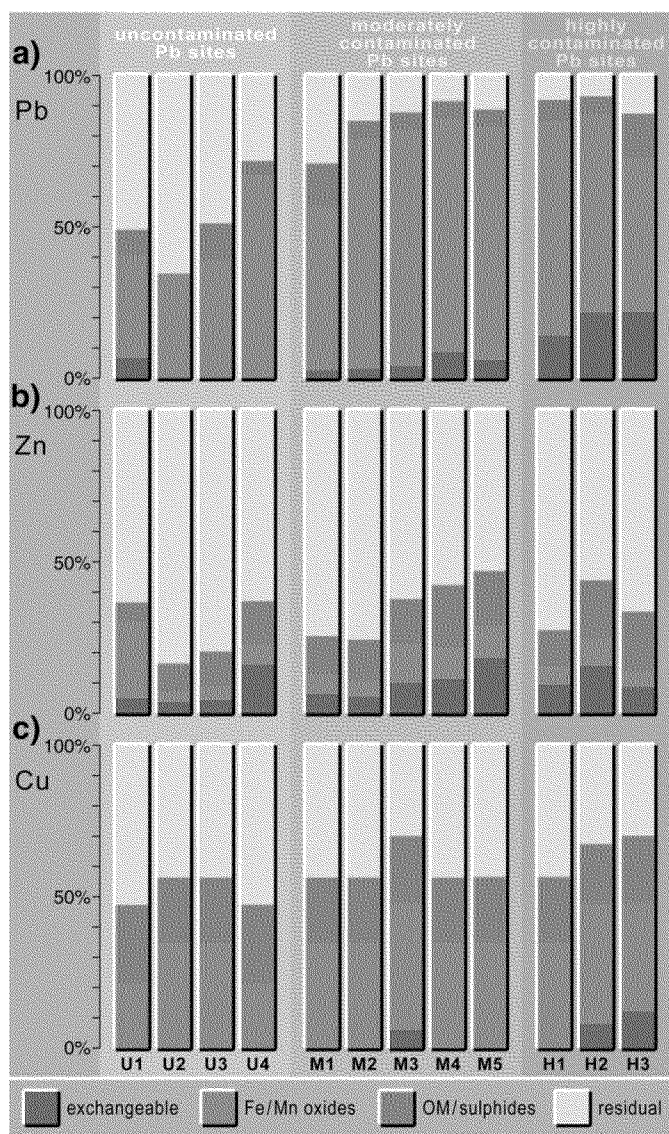


Fig. 4. Partitioning of Pb, Zn and Cu in floodplain/river terrace soils (b63 μm particle size) collected from the 12 study sites in mid-Wales.

collected from the 12 study sites are shown in Fig. 4, using the b63 μm particle size fraction as an example (we justify our concentration on this particle size on the basis that such constituents are generally associated with the highest metal concentrations, and may be consumed more by livestock than coarser particles [though more research is needed to confirm the latter observation – see later discussion]). The reducible Fe and Mn oxide phase accounted for the majority of the Pb partitioning in soils across the area (Fig. 4a). Exceptions where the residual phase dominated for soils from the control sites may be explained by some Pb concentrations being close to the limits of detection and the Fe/Mn oxide phase may be under-represented in these cases. Overall the Fe/Mn oxide phase accounted for 34–79% of soil Pb (concentrations between 16 and 1522 $\mu\text{g g}^{-1}$), in line with previous research undertaken in mid-Wales and elsewhere, so highlighting the importance of this phase in Pb partitioning (Bird et al., 2003; Bradley, 1984; Bradley and Cox, 1987; Evans, 1991; Evans and Davies, 1994; Hudson-Edwards et al., 1996; Macklin and Dowsett, 1989). However, operationally-defined by dissolution in $\text{NH}_2\text{OH} \cdot \text{HCl}$, it is possible that this phase also includes Pb from other soil fractions.

For the control and moderately contaminated soils the residual phase accounted for most of the remaining Pb (range 7–65%), but for

the most contaminated sites the readily exchangeable fraction is more important and accounted for between 6 and 22% (37–535 $\mu\text{g g}^{-1}$) of Pb in comparison to 6–14% (82–381 $\mu\text{g g}^{-1}$) associated with the residual phase. Considering all samples, the proportion of readily exchangeable Pb is strongly and significantly ($P < 0.05$) positively correlated with total Pb (Spearman rank correlation coefficient (r_s) = 0.874 and 0.976 for the b63 and 63–2000 μm fractions, respectively). As previously stated, the soils of the 12 study sites contain between 4.5 and 8.9% organic matter (median = 6.0% which can be interpreted as low; Steigmajer, 1999), and the oxidisable organic matter/sulphide phase accounted for up to 14% only of the soil Pb.

Zinc is principally associated with the residual phase, accounting for a mean 68% (min.–max. = 58–83% or 42–373 $\mu\text{g g}^{-1}$) of the total soil content (Fig. 4b). The metal is relatively evenly distributed between the other partitions: The exchangeable phase accounts for c. 10%, the Fe/Mn oxide phase for c. 8% and the oxidisable organic matter/sulphide phase for c. 14% of soil Zn. Copper exhibits a more even distribution between the phases than the other metals studied (Fig. 4c), though the low total Cu content of the soils analysed results in phase concentrations of the metal that are generally very close to or at the limits of detection, and the data should be regarded as an approximate indication of partitioning. The most reliable estimates are associated with sites M3, H2 and H3 where the mean distribution is 39%, 31% and 21% for the Fe/Mn oxide, residual and oxidisable organic matter/sulphide phases, respectively. The exchangeable Cu concentrations are generally below the detection limit except at these 3 sites where 6–12% of the total soil concentration is associated with this phase.

4. Discussion

4.1. Metal partitioning and plant availability

For the purposes of indicating plant availability, the exchangeable phase is the fraction analysed by the SEP procedure that is the form in which metals are most accessible for uptake by vegetation (e.g. Harrison et al., 1981), although this does not necessarily mean that plant concentrations always correlate with soil exchangeable content. Metals in the Fe/Mn oxide phase may also be plant available under some conditions as these oxides are moderately reducible and also thought to be relatively soluble (Li and Thornton, 2001) depending on the soil pH and oxygen conditions. In this respect the slightly/moderately acidic topsoils, and the reducing soil conditions evident at some of the study locations, are likely to promote the plant availability of the metals investigated.

Up to 22% (equivalent to 535 $\mu\text{g g}^{-1}$) of the total Pb was found to be readily exchangeable, and there is a significant correlation between the concentrations of this metal phase and those of the washed pasture herbage (e.g. Table 3), although the strength of the association is similar to those for the other phases and the sum total content. The majority (i.e. 53–83%) of Zn is associated with the least plant available residual phase, with only up to 18% of this metal linked to the

Table 3

Spearman rank correlation coefficients (r_s) determined for the association between metal concentrations in the SEP phases (and sum total content) and in washed pasture vegetation for a selected period of study (March 2000, which provides a typical example of the associations observed).

Phase	Pb	Zn	Cu
1: exchangeable	0.855 ^a	0.484	0.412
2: Fe/Mn oxides	0.876 ^a	0.313	0.271
3: oxidisable OM/sulphides	0.865 ^a	0.621 ^a	-0.037
4: residual	0.774 ^a	0.554 ^a	-0.145
Sum total of phases	0.890 ^a	0.568 ^a	0.327

The availability of metals to plants is in the phase order: exchangeable N Fe/Mn oxide bound N oxidisable OM/sulphide bound N residual.

^aDenotes a significant positive correlation at the 0.05 level.

exchangeable fraction. Correlation analysis investigating Zn concentrations in the various phases and washed herbage revealed a significant positive association for the oxidisable organic matter/sulphide and residual phases, and the sum total soil Zn concentration (e.g. Table 3). The correlation coefficients determined for the more plant available exchangeable and Fe/Mn oxide Zn phases with washed plant concentrations are weak and not significant. On the basis of the limited reliable SEP data for Cu, this metal is the least available to plants with between 6 and 12% of the total content associated with the exchangeable phase. Irrespective of soil phase (or sum total content), no significant correlation coefficient was evident between soil phase (and sum total concentration) and washed pasture plant content.

A consideration of the results obtained from the SEP indicates the complexity of the mechanisms that influence the root absorption of Pb, Zn and Cu and translocation into above-ground vegetation. Lead availability to plants is indicated with its association with the exchangeable soil phase (and the Fe/Mn oxide fraction if the observations of Li and Thornton (2001) are accepted), especially evident at sites with increased concentrations of this metal (Fig. 4), yet the relative accumulation of Pb is distinctly limited compared to Zn and Cu. Discussion and work investigating the effective barriers that limit the Zn, Cu and especially the plant–Pb concentrations of pasture herbage in mid-Wales is beyond the scope of our research, but a variety of soil (e.g. the influence of ions that can compete for absorption) and plant (e.g. genotype) factors are known to have marked effects on metal uptake and translocation (Adriano, 2001). For Zn and Cu the importance of such complicating factors result in no significant correlation between the more readily available soil phases and plant concentrations. Limitations associated with SEPs, such as the non-selective dissolution of metals within the phases, are a further aspect that needs to be considered in this context, although a number of authors have concluded that the procedures are effective for estimating plant availability and consequently they continue to be widely used in environmental risk research (e.g. Bird et al., 2003; Cao et al., 2008; Li and Thornton, 2001; Maskall and Thornton, 1998).

4.2. Soil ingestion, metal partitioning and potential availability to sheep

With some total soil–Pb contents determined in our research exceeding recommended ICRL (1990) trigger concentrations, it is clear that this is the main metal contaminant of concern to livestock farmers in mid-Wales. A dietary Pb concentration of $50 \mu\text{g g}^{-1}$ may result in accumulation of this metal in grazing animals to an extent that toxic effects may occur (Allcroft and Blaxter, 1950), or where residues in offal for human consumption exceed recommended action levels (ICRL, 1990). Whilst these concentrations were not apparent in washed pasture samples from the control sites, this limit was exceeded on many occasions from washed herbage obtained from the moderately and highly contaminated sites in the winter months (i.e. January–March). From the most highly contaminated site washed pasture concentrations exceeding $60 \mu\text{g Pb g}^{-1}$ were apparent throughout most of the year (with the exception of July and September), and the highest content recorded at this location was 14 times the $50 \mu\text{g g}^{-1}$ recommended limit. Despite the restriction in accumulation of high Pb concentrations from soils into above-ground plant material, we can conclude that there is still a risk of toxicity to grazing animals from the ingestion of soil-free plant material at sites with the most contaminated soils.

Complimenting the soil–plant–animal transfer of metals is the more direct soil–animal pathway that is especially important for Pb because of its more limited accumulation in above-ground vegetation which results in pasture being susceptible to the consequences of adhering soil particles enriched in the metal. Bearing in mind that sheep can ingest soil not just through the consumption of particles adhering to vegetation (other sources are the topsoil surface and from licking around the mouth following contact with soil), the $50 \mu\text{g g}^{-1}$

safety dietary threshold for Pb was exceeded in unwashed pasture vegetation in 28% of the samples collected overall, and all samples collected in January and March (excepting those from the uncontaminated soil control sites). Therefore, by consuming soil contaminated herbage alone (i.e. ignoring any other source of soil), grazing sheep may ingest potentially toxic concentrations of Pb across the whole study area in the winter months and throughout most of the year at the most highly contaminated sites.

Although soil ingestion is a major route of Pb to sheep in mid-Wales, any impact on livestock is dependant upon its bioaccessibility (defined as the fraction that is soluble in the animal gastrointestinal (GI) tract and is potentially available for absorption). In this respect both soil particle size and the partitioning of Pb are important. For example, a study undertaken on cattle by Allcroft (1950) found that toxic effects varied according to the size of galena: large particle PbS had a lower toxicity and resulted in a relatively reduced tissue-Pb concentration compared to that of smaller grain sizes, attributable to the fact that the latter have a greater surface area to volume ratio resulting in more rapid solubilisation in the GI tract of the animal. The topsoils of the 12 study sites exhibit a range of loam textures (e.g. stony loam, slightly gritty silty clay loam, etc., Rudeforth, 1970) and consequently by definition contain significant quantities of sand-, silt- and clay-sized particles that potentially are available for ingestion by livestock. But in considering the texture of the topsoil of the study locations, there appears to be an aspect of soil consumption that has yet to be addressed by the research community: Is the intake of particle sizes by livestock coincident with the topsoil texture, or do processes that result in the contamination of herbage with soil grains lead to a preferential intake of a particular particle size? While we do not as yet know the answer to this question, we recognise that the consumption of fine soil particles may be more significant in terms of Pb exposure than the ingestion of coarser constituents.

Generally a similarity in the partitioning of Pb, Zn and Cu was evident for each of the physical soil fractions investigated. With metals extracted earlier in the SEP being more readily soluble than those linked to the more aggressive reagents used later in the procedure, it has been suggested that such methods can be used as an inexpensive estimate of contaminant bioaccessibility (though we would argue that (a) not enough data is available to conclude that SEP is a good estimate of Pb bioaccessibility, and (b) SEP procedures are time consuming and in this respect are not inexpensive). For example, in a study directed at the human bioaccessibility of soil–Pb, Basta and Gradwohl (2000) found that the metal extracted by the initial two reagents, employed in a four-step SEP, was strongly correlated with Pb extracted by an *in vitro* procedure that replicated the intestinal phase of the digestive

Table 4

The estimated total daily Pb intakes by sheep in the form of soil compared to those associated with the exchangeable and Fe/Mn oxide phases (assumed to be the most bioaccessible forms).

Sites	Intake as soil (mg d^{-1})		
	Exchangeable phase	Fe/Mn oxide phase	Total intake
U1	b1	1–2	2–5
U2	b1	b1–3	1–8
U3	b1	b1–10	b1–21
U4	b1	b1–11	b1–16
M1	b1–1	1–14	1–24
M2	b1–3	1–66	2–89
M3	1–9	12–168	16–215
M4	2–18	16–161	20–207
M5	b1–6	4–8	5–113
H1	6–52	32–288	45–400
H2	7–334	23–1046	34–1580
H3	3–266	9–680	17–1292

The ranges given represent intakes throughout the year, and are calculated from the soil ingestion rates determined in our study.

system (i.e. the part of the human GI environment where absorption of elements mainly occurs). If it is assumed that Pb associated with the exchangeable and Fe/Mn oxide soil phases determined in our research is potentially bioaccessible to sheep, then taking the recorded rates of soil ingestion that we have found into account, we estimate that the livestock grazing the floodplain sites of mid-Wales can ingest over 300 mg Pb d⁻¹, and greater than 1000 mg Pb d⁻¹, of the metal associated with these exchangeable and Fe/Mn oxide soil fractions, respectively (Table 4). Sheep at five sites (M3, M4, H1, H2 and H3) were found to be periodically ingesting soil–Pb concentrations linked to these phases in excess of the 60–100 mg d⁻¹ guidelines recommended by the ARC (1980) for animal health. In making such observations we recognise the complexities of the ovine GI tract – where metals may potentially be absorbed at a range of sites from the rumen to the small intestine; Wilkinson et al. (2003) – and appreciate that the extent to which Pb associated with these two soil phases is truly bioaccessible remains unknown. Nevertheless, the indications are that significant quantities of relatively soluble soil–Pb can be ingested by sheep grazing the moderately and highly contaminated floodplain pastures of mid-Wales.

5. Conclusions

Within and downstream of the metalliferous area of mid-Wales, floodplain soils are contaminated with elevated concentrations of Pb, Cu and Zn attributable to the weathering of mineral ores, historical mining activity and fluvial redistribution. In particular soil–Pb at some sites exceeds 'trigger' concentrations and presents a risk of toxicity to grazing livestock. The results obtained by subjecting soils to a SEP suggest a ready bioavailability of Pb to pasture plants, especially at the contaminated sites, with up to 22% of the total content of this metal associated with the exchangeable phase. But whilst metal concentrations in pasture vegetation reflect the high concentrations in the soils, uptake and translocation into the above-ground plant tissues is limited, especially for Pb, for which a variety of soil and/or plant mechanisms are thought to be effectively responsible. The low relative accumulation of Pb in pasture plants compared to the high degree of soil contamination of vegetation surfaces evident, especially in the winter months, increases the importance of the direct soil–animal pathway in the exposure of grazing sheep to the elevated soil–Pb concentrations. High soil ingestion rates reflect this with a clear seasonal pattern of increase over the winter months when sheep may ingest up to half (and occasionally potentially more) of their DM intake, and nearly 100% of Pb intake, in the form of soil. Whilst knowledge on the bioaccessibility of this metal is limited, the SEP indicates that significant quantities of relatively soluble soil–Pb are ingested by sheep grazing moderately and highly contaminated pastures. Small particle sizes in particular may be an important source of Pb exposure to grazing livestock, but no research has as yet focussed on investigating the actual soil grain or aggregate sizes that are consumed, or their fate within the animal GI tract.

Since little research has been conducted to date on the bioaccessibility of ingested soil-borne contaminant metals and the extent to which these elements are absorbed into the body of grazing stock, our research is now directed towards answering these questions. In the meantime our study has shown that in mid-Wales it is desirable to reduce the intake of ingested soil–Pb by improving pasture quality and availability. This may be achieved in a variety of ways such as utilisation of an effective rotation of livestock, a reduction of the stocking rate and/or provision of supplementary feed during the winter months.

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